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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

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First Inventor or Application Identifier	Mytych et al.
Title	Methods and Reagents for the Detection of Antibodies etc
Express Mail Label No.	EL226883618US

APPLICATION ELEMENTS
See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Assistant Commissioner for Patents
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1. ☒ * Fee Transmittal Form *(e.g., PTO/SB/17)*
   (Submit an original and a duplicate for fee processing)

2. ☒ Specification [Total Pages 36]
   (preferred arrangement set forth below)
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure

3. ☐ Drawing(s) *(35 U.S.C. 113)* [Total Sheets]

4. Oath or Declaration [Total Pages]
   a. ☐ Newly executed (original or copy)
   b. ☐ Copy from a prior application (37 C.F.R. § 1.63(d))
      (for continuation/divisional with Box 16 completed)
      i. ☐ DELETION OF INVENTOR(S)
      Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).

NOTE FOR ITEMS 1 & 13: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28).

5. ☐ Microfiche Computer Program *(Appendix)*

6. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. ☐ Computer Readable Copy
   b. ☐ Paper Copy (identical to computer copy)
   c. ☐ Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

7. ☐ Assignment Papers (cover sheet & document(s))
8. ☐ 37 C.F.R.§3.73(b) Statement ☐ Power of *(when there is an assignee)* Attorney
9. ☐ English Translation Document *(if applicable)*
10. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
11. ☐ Preliminary Amendment
12. ☒ Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
13. ☐ * Small Entity Statement(s) (PTO/SB/09-12) ☐ Statement filed in prior application, Status still proper and desired
14. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
15. ☒ Other: Cover Sheet for Patent Applic. Declaration unexecuted 4 pgs

16. **If a CONTINUING APPLICATION**, *check appropriate box, and supply the requisite information below and in a preliminary amendment:*
   ☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No: _____/_____
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   For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation <u>can only</u> be relied upon when a portion has been inadvertently omitted from the submitted application parts.

17. CORRESPONDENCE ADDRESS

☐ Customer Number or Bar Code Label | 24265 *(Insert Customer No. or Attach bar code label here)* | or ☒ Correspondence address below

Name	Donald W. Wyatt
	Patent Dept. K-6-1, 1990
Address	Schering-Plough Corporation
	2000 Galloping Hill Road

| City | Kenilworth | State | New Jersey | Zip Code | 07033-0530 |
| Country | USA | Telephone | 908-298-2902 | Fax | (908) 298-5388 |

| Name *(Print/Type)* | Donald W. Wyatt | Registration No. *(Attorney/Agent)* | 40879 |
| Signature | | Date | August 22, 2000 |

PATENT APPLICATION

METHODS AND REAGENTS FOR THE DETECTION
OF ANTIBODIES TO ADENOVIRUS

INVENTORS:

Daniel T. Mytych, a citizen of the United States, residing at 3162 Saddleback Court, Thousand Oaks, CA 91360, U.S.A.

Steven J. Swanson, a citizen of the United States, residing at 4318 Timberdale Road, Moorpark, CA 93021, U.S.A.

ASSIGNEE: **Schering Corporation**

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Date of Deposit:___August 22, 2000_____

Donald W. Wyatt
Schering-Plough Corporation
Patent Department, K-6-1, 1990
2000 Galloping Hill Road
Kenilworth, New Jersey 07033-0530
Telephone No.:   (908) 298-2902
Facsimile No.:   (908) 298-5388

METHODS AND REAGENTS FOR THE DETECTION
OF ANTIBODIES TO ADENOVIRUS

5   This application claims the benefit of United States Provisional Application

No. 60/150,622 filed August 25, 1999.

Throughout this disclosure, various publications, patents and patent applications are

referenced. The disclosures of these publications, patents and patent applications are herein

incorporated by reference.

10   **Field of the Invention**

The present invention pertains to methods and reagents for the detection of

antibodies that bind to adenovirus. In preferred embodiments, the present invention is

directed to detecting antibodies to adenovirus 5. The usefulness of the present

invention includes, but is not limited to, identifying patients likely to have an immune

15   response to gene therapy, vaccines to prevent or treat adenovirus infection, therapeutics

to induce passive immunity to adenovirus infection and the like.

Background

Prospects for gene therapy to correct genetic disease or to deliver therapeutic

molecules depend on the development of gene transfer vehicles that can safely deliver

20   exogenous nucleic acid to a recipient cell. To date, most efforts have focused on the

use of virus-derived vectors that carry a heterologous gene (transgene) in order to

exploit the natural ability of a virus to deliver genomic content to a target cell.

For example, despite their reputation as major pathogenic agents that lead to

numerous infectious diseases, adenoviruses (and particularly, replication-deficient

25   adenoviruses) have attracted considerable recognition as highly effective viral vectors

for gene therapy. Adenoviral vectors offer exciting possibilities based on their high

efficiency of gene transfer, substantial carrying capacity, and ability to infect a wide

range of cell types. Due to these desirable properties of adenoviruses, recombinant

adenoviral vectors have been used for the cell-targeted transfer of one or more

30   recombinant genes to diseased cells or tissue in need of treatment. In fact, adenovirus-

based vectors offer several advantages, including tropism for both dividing and non-

dividing cells, minimal pathogenic potential, ability to prepare vector stocks at high

titer, and the potential to carry large DNA inserts. To date, genes that have been

expressed by adenoviral vectors include p53, dystrophin, erythropoietin, ornithine

transcarbamylase, adenosine deaminase, interleukin-2 and α-antitrypsin. Examples of adenovirus vectors can be found in U.S. Patent No. 5,585,362 to Wilson et al., U.S. Patent No. 5,824,544 to Armentano et al., and U.S. Patent No. 5,846,782 to Wickham et al.

5   One barrier to successful gene transfer by viral vectors to patient hosts is the immune response of the host to the introduction of the virus. In terms of the general structure of an adenovirus, under the electron microscope, an adenovirus particle resembles a space capsule having protruding antennae. The viral capsid comprises at least six different polypeptides, including hexon, base and fiber proteins. The fiber,

10   together with the hexon (Crawford-Miksza, L. and Schnurr, D. P., J. Virology, Mar 1996, pp.1836-1844), determine the serotype specificity of the virus, and also comprise the main antigenic determinants of the virus.

        This ability of adenoviral fiber and hexon protein to act as targets for a host immune response hamper attempts at adenoviral-mediated gene therapy. Namely,

15   following adenoviral vector re-administration to prolong the therapeutic response, neutralizing antibodies develop against the adenoviral fiber and/or hexon proteins, thus circumventing adenoviral gene delivery to host cells. As the therapy is expensive, it is extremely wasteful to utilize a viral vector for gene therapy in a patient host that will mount an immune response to the vector.

20   What is needed is an efficient method of determining the likelihood that a patient host's immune system will interfere with intended gene therapy using viral vectors.

Summary of the Invention

        The present invention provides methods and reagents for detecting antibodies

25   capable of binding to adenovirus. In one embodiment, the present invention provides a method for detecting antibodies capable of binding to adenovirus comprising: a) immobilization of one or more peptides capable of being bound by an anti-adenovirus antibody directly onto a flowcell of a sensorchip in a biosensor, b) obtaining a serum sample from a patient to be tested and contacting said serum sample with the

30   immobilized peptide, and c) measuring binding of antibodies to the immobilized peptide by means of the biosensor. In preferred embodiments, more than one peptide is utilized and are directly immobilized, each on its own separate flowcell.

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In another embodiment, the present invention provides a method for detecting antibodies capable of binding to adenovirus, comprising a peptide selected from the group consisting of: AATALEINLEEEDDDNEDEVDEQAEQQKTHVF-Amide, IGVEGQTPKYADK-Amide, YETEINHAAGRVLKK-Amide, GILVKQQNGKLESQ-Amide, STTEATAGNGDNLTPKV-Amide, MPTIKEGNSRELMG-Amide, VINTETLTKVKPKTGQENGWEKDATEFSDK-Amide, or peptides having substantial sequence identity thereto. While not limited to a specific method of detection, in one embodiment the method of detecting comprises an ELISA system.

In yet another embodiment, the present invention provides a composition of matter comprising CKGKG or a peptide having substantial sequence identity thereto, and their use in a biosensor based assay to detect antibodies.

While certain embodiments of the present invention is not limited to specific peptides, in preferred embodiments the peptide is capable of being bound by antibodies specific to adenovirus 5. Examples of such peptides include those described above, as well as the following: CKGKGAATALEINLEEEDDDNEDEVDEQAEQQKTHVF-Amide, CKGKGIGVEGQTPKYADK-Amide, CKGKGYETEINHAAGRVLKK-Amide, CKGKGGILVKQQNGKLESQ-Amide, CKGKGSTTEATAGNGDNLTPKV-Amide, CKGKGMPTIKEGNSRELMG-Amide, CKGKGVINTETLTKVKPKTGQENGWEKDATEFSDK-Amide, or peptides having substantial sequence identity thereto.

In another embodiment, the present invention encompasses peptides that correspond to family reactive determinants of hexon and penion. This includes peptides that cross-react with similar antigens of other viral vectors.

The present invention is also not limited by the sample to be analyzed for antibodies. In one embodiment, the sample is human serum. In a preferred embodiment, the serum sample is simultaneously contacting with a plurality of immobilized peptides. Likewise, it is preferred that when more than one peptide is used, at least one of the plurality of peptides is capable of being bound by antibodies specific to adenovirus 5.

While not limited by the desired results of an assay, in preferred embodiments, the amount of antibody that binds to each peptide is directly proportional to the response units that are reported by the biosensor.

Finally, although the present invention is not limited by the equipment used; in a preferred embodiment, it is a BIACORE 2000™ biosensor.

(The above sequences are listed using standard one-letter amino acid symbols; *see e.g.*, Lehninger, *Principles of Biochemistry*, Worth Publishers, Inc. 6th Ed. 1988, p.96).

Definitions

5   An "antibody" includes, but is not limited to, immunoglobulin molecules and immunologically active portions of immunoglobulin molecules such as portions containing a paratope (i.e., an antigen binding site). In particular, an antibody preferably can be a bispecific antibody, i.e., having one paratope directed to an epitope of the chimeric fiber protein, and another paratope directed to an epitope of a cell surface-binding site.

10   A "vector" according to the invention is a vehicle for gene transfer, as that term is understood by those skilled in the art. Three types of vectors encompassed by the invention are plasmids, phages, and viruses. Plasmids, phages, and viruses can be transferred to a cell in their nucleic acid form (e.g., via transfection). In comparison, phages and viruses also can be transferred with the nucleic acid in a "capsular" form.

15 Hence, the vectors (e.g., capsular form) that can be employed for gene transfer are referred to herein generally as "vectors", with nucleic acid forms being referred to more particularly as "transfer vectors". However, transfer vectors also are vectors within the context of the invention.

         "Substantial sequence identity" means that two peptide sequences, when

20 optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity or more. Preferably, residue positions that are not identical differ by conservative amino acid substitutions. For example, the substitution of amino acids having similar chemical

25 properties such as charge or polarity are not likely to effect the properties of a protein. Examples include glutamine for asparagine or glutamic acid for aspartic acid. In addition, substantial sequence identity encompasses amino acids or chemicals that create a similar structure. For example, linear, cyclic, or constrained peptides.

Detailed Description of the Invention

30   The present invention provides methods and reagents for detecting antibodies to adenovirus. In one embodiment, the present invention provides peptides for detecting anti-adenovirus antibodies. In another embodiment, the present invention provides novel peptides for detecting anti-adenovirus antibodies in a biosensor-based assay. The

present invention is not limited to the serotype of the adenovirus capable of being bound by the antibodies detected. In a preferred embodiment, however, the serotype is adenovirus 5 (AV5).

5   The present invention is also not limited to the specific type of assay used to detect the anti-adenovirus antibodies.

In one embodiment, the present invention contemplates the use of an enzyme-linked immunosorbant assays (ELISA). An ELISA assay, in general, is performed by binding a reference reagent (antigen) to a solid phase support. Samples to be tested are mixed with a labeled reagent, then reacted with the bound reference reagent. The

10   reagents then undergo a series of dilution, incubation, and washing steps in order to separate bound and free reagents. The process concludes with a detection step, compatible with the type of label used, designed to indirectly measure the amount of antibody (or antigen) in the samples tested. For example, a commercially available enzyme-linked immunosorbant assay (ELISA) to detect enteric adenoviruses based on a

15   polyclonal antibody to enteric adenovirus hexon protein is available (Adeno-Type 40/41 EIA, Cambridge Bioscience, Cambridge, MA).

In another embodiment, the present invention contemplates the use of ORIGEN electrochemilumninescence (ECL) detection system (Igen, Gaithersburg, MD). Briefly, the ECL system utilizes strepavidin-coated paramagnetic beads to capture and detect

20   immune complexes formed between antigen bound to a biotin-conjugated antibody.

The present invention also contemplates the use of a biosensor-based assay. The biosensor used in the present invention is preferably a BIACORE 2000™ from Pharmacia (Uppsalla, Sweden). The BIACORE 2000™ operates on a principle of surface plasmon resonance and allows for high throughput analyses. (*See, e.g.,*

25   Hodgson, Bio/Technology vol. 12, Jan. 1994 for a review of biosensors). Other biosensors, *e.g.,* a BIACORE™ X (Biacore 1000™; Biacore 3000™) from Pharmacia or an IAsys biosensor from Fisons, could be used in connection with the present invention. In analyzing the results of the assay, it is preferred that the amount of antibody that binds to each peptide is directly proportional to the biosensor signal (*e.g.,* the response

30   units that are reported by the BIACORE 2000™ biosensor).

Among other advantages, use of a biosensor assay in the present invention provides an assay that is suited for detecting lower affinity antibodies (Obenauer-Kutner et al., Journal of Immunological Methods, 1997, 206:25-33; Swanson et al., Dev. Biol. Stand. Basel, Karger, 1999, 97:135-147). Methods employing ELISAs have

difficulty in this regard because ELISAs require multiple incubation steps followed by wash cycles during which lower affinity antibodies can wash away. There is also a need for an assay that is less labor intensive than the methods utilizing an ELISA format. Finally, there is a need to significantly reduce analysis time, preferably to less

5 than 10 minutes/sample, to allow for high throughput analyses. As mentioned above, ELISAs require multiple incubation steps followed by wash cycles, and therefore the materials used in the assay cannot be regenerated for analysis of subsequent samples.

While the present invention is not limited to specific peptides for detection of adenovirus, preferred peptides for use in the methods of the present invention are

10 comprised of one or more of the sequences below (or peptides having substantial sequence identity thereto):

   a)   Peptide SEQ ID NO: 1 (HVR1 of AV5),

          AATALEINLEEEDDDNEDEVDEQAEQQKTHVF-Amide;

   b)   Peptide SEQ ID NO: 2 (HVR2 of AV5),

15   IGVEGQTPKYADK-Amide

   c)   Peptide SEQ ID NO: 3 (HVR3 of AV5),

          YETEINHAAGRVLKK-Amide

   d)   Peptide SEQ ID NO: 4 (HVR4 of AV5),

          GILVKQQNGKLESQ-Amide

20   e)   Peptide SEQ ID NO: 5 (HVR5 of AV5),

          STTEATAGNGDNLTPKV-Amide

   f)   Peptide SEQ ID NO: 6 (HVR6 of AV5),

          MPTIKEGNSRELMG-Amide

   g)   Peptide SEQ ID NO: 7 (HVR7 of AV5),

25   VINTETLTKVKPKTGQENGWEKDATEFSDK-Amide

The above sequences are described in Crawford-Miksza et al., J. Vir. 70:1836, 1996, Toogood et al., J. Gen. Vir. 73:1429, 1992, and Kinloch et al., J. Biol. Chem 259:6431, 1984. These peptides, however, are reported as part of conformational proteins. Surprisingly, peptides HVR1 (SEQ ID NO: 1) and HVR6 (SEQ ID NO: 6)

30 have been identified as part of internalized loops within the hexon protein that are not even exposed to the external surface (Haase & Pereira, J. Immunol. 108:633-636, 1972; Kjellen & Pereira, J. Gen. Vir. 2:177-185, 1968; Mautner & Wilcox, J. Gen Vir. 25:325-336, 1974; Norrby, Virology 37:565-576, 1969; Wilcox & Mautner, J. Immunol. 116:19-24, 1976). It would be expected, therefore, that if these peptides are

suitable as targets for antibody binding, that the target of antibody binding is part of the protein's overall secondary and tertiary structure. As such, it is surprising that these short linear peptides, even within the internal loop, are suitable for detecting neutralizing viral antibodies. Furthermore, it is surprising to find serotype-specificity

5   within peptides that include conserved sequences between serotypes.

When a peptide is used in a biosensor-based assay, it is preferred that a positively charged leader sequence be added next to a cysteine at the beginning of the peptide. This leader sequence interacts with the net negatively charged solid support of the biosensor system to enhance the immobilization of the peptide to the carboxymethyl

10   dextran support surface. While not limited to a particular leading sequence, the first position must be cysteine (C) and the second position can be either lysine (K), arginine (R) or histidine (H), in a preferred embodiment, the leading sequence is CKGKG (SEQ ID NO: 8) (or peptides having substantial sequence identity thereto). This results in preferred peptides for use in a biosensor-based assay of (or peptides having substantial

15   sequence identity thereto):

a)   Peptide SEQ ID NO: 9 (HVR1 of AV5),
CKGKGAATALEINLEEEDDDNEDEVDEQAEQQKTHVF-Amide;

b)   Peptide SEQ ID NO: 10 (HVR2 of AV5),
CKGKGIGVEGQTPKYADK-Amide

20   c)   Peptide SEQ ID NO: 11 (HVR3 of AV5),
CKGKGYETEINHAAGRVLKK-Amide

d)   Peptide SEQ ID NO: 12 (HVR4 of AV5),
CKGKGGILVKQQNGKLESQ-Amide

e)   Peptide SEQ ID NO: 13 (HVR5 of AV5),
25   CKGKGSTTEATAGNGDNLTPKV-Amide

f)   Peptide SEQ ID NO: 14 (HVR6 of AV5),
CKGKGMPTIKEGNSRELMG-Amide

g)   Peptide SEQ ID NO: 15 (HVR7 of AV5),
CKGKGVINTETLTKVKPKTGQENGWEKDATEFSDK-Amide

30

When viral vectors are used to deliver gene therapy to a patient, the patient's immune reaction to the viral vector has a significant impact on the success of the therapy. For example, if a patient's immune system produces antibodies to the viral

vector, the vector may be inactivated before it has properly delivered the therapeutic gene to the patient's cells.

Therefore, information about a patient's immune system and their antibodies against viral vectors is important. As an example, the level of high-concentration and/or high-affinity antibodies will have a direct impact on therapy. Moreover, the level of low-concentration and/or low-affinity antibodies is also an important factor to consider. Even though the amount of low-affinity antibodies may have a limited impact on the early stages of therapy, the data obtained may predict the development of increased quantities of antibodies or additional higher-affinity antibodies. Furthermore, the ratio of high-affinity to low-affinity antibodies is a valuable indicator that permits a medical provider to design and monitor a patient's therapy.

When the methods of the present invention are used, it is possible to detect low-concentration and/or low-affinity antibodies and differentiate them from the high concentration and/or high affinity antibodies. For example, using a system such as the BIACORE 2000™ to detect antibodies provides information on the amount of antibodies that can bind to an antigen, whether they are high- or low-affinity antibodies. This information can be compared to detection of antibodies under more stringent conditions (e.g., ELISA) that detect high-concentration and high-affinity antibodies. Thus an immune status profile can be generated that evaluates the amount of total antibodies, and whether they are of high-affinity and/or high concentration antibodies or low-affinity and/or low concentration antibodies.

The present invention further contemplates the preparation and use of a vaccine composition for the treatment of human adenovirus infection, including AV5. The preparation of such a vaccine is accomplished by utilization of at least one of the above peptides. This can be accomplished by utilization of at least one of the above peptides. This can be accomplished by genetic engineering of at least one of the above peptides and expressing at least one of these proteins in suitable vector/host cell systems such as bacteria, yeast or any other suitable vector/host system. In another embodiment, the present invention is directed to a Type II vaccine which is a combination of an inactivated adenovirus and at least one of the above-listed proteins.

By vaccine is meant an agent used to stimulate the immune system of a living organism so that protection against future harm is provided. A number of viral polypeptide preparations derived from viral coats or envelopes have been suggested as possible active components for vaccine compositions. For example, U.S. Pat. No.

4,470,967 describes vaccine preparations which are made by complexing viral polypeptide with a lectin, the latter element acting as adjuvant. A number of references, (e.g., 4,344,935 or 4,356,169 or Morein, et al., J. Gen. Virol., 64: 1557-1569, 1983), utilize a method of preparing parainfluenza glycoprotein compositions in

5 which the viral glycoprotein HN and F are solubilized with a detergent, to extract them from the viral envelope, followed by some method of phase separation in order to remove the detergent and lipids. The latter procedures produce a glycoprotein subunit that is not only substantially detergent-free, but also lipid-free. The latter type of highly purified glycoprotein is considered the preferred type of active agent for potential use of

10 commercial vaccine. An alternative approach is the synthesis of peptides for use as a vaccine.

Recombinant DNA techniques for the preparation of recombinant adenovirus peptides for use in the preparation of vaccines are sufficiently well known and widespread so as to be considered routine. In very general and broad terms, a method

15 for use herein consists of transferring the genetic material, or more usually part of the genetic material, of one organism into a second organism so that the transferred genetic material becomes a permanent part of (recombines with) the genetic material of the organisms to which it is transferred.

This usually consists of first obtaining a small piece of DNA from the parent

20 organism either from a plasmid or a parent chromosome. A plasmid (also called an extrachromosomal element) is a hereditary unit that is episomal, (i.e.- physically separate from the chromosome of the cell). The DNA may be of any size and is often obtained by the action of a restriction endonuclease enzyme that acts to split DNA molecules at specific base-pair sites. In the present invention an adenovirus peptide

25 gene can be synthesized based upon the sequence of the protein to be expressed.

DNA pieces may be transferred into a host cell by various means. For example, transformation wherein naked DNA is internalized into a cell from the external environment, oftentimes through artificially induced disruption of the cell membrane (e.g. by introduction of various chemical agents, such as calcium ions or lipids, by

30 application of electric current, by extreme temperature changes or by microinjection). Other methods of gene transfer such as transduction are also suitable, wherein DNA is packaged within a phage such as a cosmid or viral vector.

10

Once DNA is in the host cell, it may continue to exist as a separate piece (generally true of complete transmitted plasmids) or it may insert into the host cell chromosome and be reproduced together with the chromosome during cell division.

Administration of a vaccine contemplated by the present invention to the patient (human or animal) may be by any known or standard techniques. These include oral ingestion, intestinal intubation, or broncho-nasal spraying. Other methods of administration, such as intravenous injection, that allow the carrier microbe to reach the human or animal's bloodstream may be acceptable when the carrier microbe is replication deficient.

The amount required will vary with the antigenicity of the gene product and need only be an amount sufficient to induce an immune response typical of existing vaccines. Routine experimentation will easily establish the required amount. Typical initial dosages of vaccine could be about 0.001-100 mg antigen/kg body weight, with increasing amounts or multiple dosages used as needed to provide the desired level of protection.

The pharmaceutical carrier in which the vaccine is suspended or dissolved may be any solvent or solid that is non-toxic to the inoculated animal and compatible with the carrier organism or antigenic gene product. Suitable pharmaceutical carriers include liposomes and liquid carriers, such as normal saline and other non-toxic salts at or near physiological concentrations, and solid carriers, such as talc or sucrose. Adjuvants, such as Freund's adjuvant, complete or incomplete, may be added to enhance the antigenicity via the bronchial tubes, wherein the vaccine is suitably present in the form of an aerosol. Booster immunizations may be repeated numerous times with beneficial results.

In another aspect, the present invention relates to a method of treating infectious diseases caused by adenovirus infection by introducing passive immunity. Passive immunization, as defined herein, refers to resistance (e.g., temporary or sustained protection against infection) based on giving preformed antibodies to a patient from an in vivo or in vitro source. The main advantage of passive immunization is the prompt availability of large amounts of antibodies against human adenoviruses that can be identified as described in the above embodiment of the present invention.

For example, the present invention also encompasses antibodies, either monoclonal or polyclonal, that are identified using the systems described above and are useful in the therapeutic control of infection by adenoviruses. Said antibodies can be

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prepared by injecting mammalian species, e.g., human, horse, rabbit, sheep, mice, etc. with the peptides described above and then purifying said antibodies employing the detection systems contemplated and described herein.

In another embodiment, the present invention relates to the development of

5   specific human or other eukaryotic (e.g., yeast, baculovirus, or Chinese hamster cells) polyclonal or monoclonal antibodies, as well as human-mouse chimeric polyclonal or monoclonal antibodies for administration in passive immunization against human adenoviruses. Such antibodies can be used as created in these systems, or they can be humanized. Methods of humanizing antibodies is described in U.S. Patents No.

10   5,597,710 and 5,705,154 to Dalie et al. and U.S. Patent No. 5,585,089 to Queen et al.

In addition, the generation of antibodies against the disclosed peptides can be used for commercial use. In one embodiment, these antibodies may be used as probes for various biochemical assays to detect adenovirus, more preferably AV5. In another embodiment, these antibodies may be used in kits to detect IR.

15   The present invention further contemplates the use peptides as probes to detect, by hybridization, cellular DNA from infected tissue (e.g. biopsy material) carrying integrated structural adenovirus DNA (i.e., DNA encoding one of the peptides described above). The probe can be DNA, cDNA, recombinant DNA or RNA.

In one particular embodiment of the present invention, the probes may be used

20   for in situ hybridization. For example, patient specimens (tissue or tissue extracts) containing biopsy material are smeared onto a standard microscope slide, then fixed with an appropriate fixative. The DNA or RNA probe, which has been labeled (e.g. with biotin-avidin-enzyme) is added. The slide is then placed onto a heating block for one or two minutes to allow both the probe and the target nucleic acids to be separated

25   from their complementary strand (if double stranded). Non-hybridized probe DNA or RNA is removed by gentle washing. After a suitable detection complex is added, hybridization is detected with a light microscope following formation of a colored compound. In other embodiments of the invention, the nucleic acid probe is labeled with a radioactive isotope or chemiluminescent tag. Alternatively, tissue to be tested

30   may be lysed and DNA/RNA fixed to, nitrocellulose paper for example. Hybridization and DNA/RNA detection systems are well known in the art.

The following examples serve to illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

Example 1:   MATERIALS AND METHODS

1.1   Equipment and Materials

5   BIACORE 2000™ biosensor instrument, amine coupling kit (NHS/EDC), PDEA, sensorchip CM5 (research grade) and P-20 surfactant were all obtained from BIACORE™, Uppsala, Sweden. The HEPES, NaCl, EDTA, Tris and 0.2µM filters were obtained from Fisher Scientific, Springfield, New Jersey. The cysteine was obtained from Aldrich, Milwaukee, Wisconsin.

10   The carboxymethyldextran was obtained from Fluka Chemical Corp., Ronkonkoma, New York.

1.2   Peptide design

15   Each peptide was designed to contain an amino-terminal cysteine residue available for thiol-coupling followed by a KGKG linker to each of the seven hypervariable region (HVR) sequences of adenovirus type 5 hexon. The linker is designed to function as a spacer, as well as to provide a localized positive charge around the cysteine; thus facilitating thiol-coupling by attraction to the negatively charged sensorchip matrix. Each of the seven unique hypervariable sequences

20   corresponding to adenovirus type 5 hexon was obtained from published sequences (Crawford-Miksza and Schnurr, 1996). These amino acid sequences are displayed according to the single letter code in Table 1. All seven linear peptides were synthesized by Research Genetics, Huntsville, Alabama and subsequently purified by HPLC to 85% purity.

25

1.3   Method of biosensor peptide immobilization

        Using the BIACORE 2000™, each of the seven peptides was immobilized onto a flow cell contained on a sensorchip. All peptides were diluted to 500µg/ml. HVR 1, 2, 4, and 5 were diluted with 10mM MES pH 5 and HVR 3, 6 and 7 were diluted in

30   10mM sodium acetate buffer pH 4. Thiol-coupling chemistry was the standard coupling chemistry used for all peptides. The immobilization procedure was performed at a continuous flow rate of 5µl per minute followed by the sequential injection of 20µl of NHS/EDC, 40µl of PDEA, 50µl of peptide, and 40µl of cysteine/1M NaCl. Each immobilized peptide was subsequently restored with a 5µl pulse of 50mM HCl between

35   sample analyses.

1.4   Biosensor analysis of serum samples:

Interaction of antibodies with Ad5 HVR peptides was monitored using a
biosensor instrument (BIACORE 2000™). All BIACORE analysis was preformed at 25
°C using 10mM HEPES buffered saline (HBS) with 0.05 % P-20, and 3.4mM EDTA
(HBS) as the BIACORE running buffer. Specifically, the BIACORE running buffer

5   consisted of 10mM HEPES, 150mM NaCl, 3.4mM EDTA, and 0.05% P-20 pH 7.4.
The HBS was filtered using 0.2μm filter and subsequently de-gassed for 30 minutes at
25°C. Sample diluent contained HBS with 1 mg/ml soluble carboxymethyldextran
(CM-D).

Serum sample analysis was performed by diluting serum 1:20 in HBS w/P-20

10   and CM-D, filtering through a 0.2μm filter and then testing a 20μl aliquot of each
sample for binding to each of the immobilized peptides at a flow rate of 5μl/minute.
This protocol was followed for analysis of rats, rabbits and pigs immunized with rAd5,
swine samples from a pre-clinical study as well as human samples from a clinical study.
In addition, commercial type-specific pAb and mAbs, diluted based on antibody purity

15   (antisera, purified IgG, etc.) were also tested for their reactivity to the HVR peptides.
An automated method was run and data collected. The binding of each sample was
recorded in response units (RU).

1.5 Affinity purification of anti-HVR1 peptide antibodies

20   HVR1 peptide was covalently coupled to a Sulfo-link agrose gel (Pierce,
Rockford, Illinois). Approximately 2.5 ml of gel was equilibrated with binding buffer
(50mM Tris, 5mM EDTA pH 8.5), centrifuged at 500 X g for 5 minutes and
supernatant subsequently decanted. A total of 2.8mg of HVR1 peptide was mixed with
2.0ml of gel in a total volume of 2ml buffer for 2 hours at room temperature on a

25   Nutator. (Note: Mixture is light-sensitive, therefore must be covered with foil). The
slurry was centrifuged at 500 X g for 5 minutes, and 2 ml of supernatant was removed.
The gel was washed 5-6 times with 1 ml aliquots of binding buffer. The gel was then
blocked by mixing it with 2 ml of 50mM cysteine for 45 minutes at room temperature.
The slurry was centrifuged, liquid decanted, and the gel equilibrated with 4 ml of

30   binding buffer and stored at 4 °C.

A 1.0 ml aliquot of serum from patient IK taken 14 days after receiving rAd5
was mixed with 1.0 ml of phosphate buffered saline (PBS), filtered through a 0.22 μm
microcentrifuge tubes. The 2.0ml of diluted serum sample was then added to 0.5 ml of
gel and mixed overnight on a nutator at 4°C. The slurry was then transferred to a

35   disposable column. The column containing the gel was then washed with 10 ml of
PBS. Bound antibodies were then eluted by addition of 2.0ml aliquots of 100 mM
Glycine-HCL pH 2.8 and 0.5ml fractions were collected. Each eluted fraction was
neutralized by the addition of 12.5μl of 2M Tris pH 11 to each fraction. All fractions

14

were then dialyzed against 4 liters of PBS using 10 KD MWCO Slide-a-lyzer cassettes from Pierce. Protein determination of IK (fraction 1) was determined using a Pierce BCA Kit following the manufacturer's instructions.

5   1.6   Neutralization of anti-peptide HVR1 antibody

In this assay, a constant amount of rAd5 is spiked into diluted serum samples. The spiked serum samples are then added to a monolayer of 293 cells grown on 6 well plates. The "control" consists of 293 cells infected with the rAd5 alone, in the absence of the serum. After a 48 hours infection, the cells are trypsinized, washed and

10   permeabilized, and bound to a FITC labeled antibody to rAd5 hexon. The cell suspension is passed through a Flow Cytometer, and the fraction of fluorescent cells is enumerated. The spiked rAd5 in each sample infects the 293 cells and produce high levels of hexon protein. This protein will bind to the antibody and yield a positive fluorescent signal in the Cytometer. The more infectious virus present in the samples,

15   correlates with a greater # of fluorescing cells. Therefore, a sample containing SNFs to rAd5 will inhibit the infectivity and thus reduce the number of positive fluorescing cells compared to control. A series of dilutions of each serum sample was used and the dilution that gives 50% fluorescence compared to control determines the titer of the SNFs in the serum sample.

20

1.7   Immune Serum preparation

Rabbits, pigs and rats were immunized with a highly purified, non-replicating rAd5 (Schering-Plough, Kenilworth, N.J.). Two Yorkshire pigs were immunized subcutaneously every four weeks with 200μg of rAd5 in 1.0ml of RIBI adjuvant (RIBI

25   Biologicals) as a primary boost followed by maintenance boosts of 100μg of rAd5 in 1.0ml of RIBI thereafter every month for several years. The six Sprague Dawley rats and two NZW rabbits were immunized subcutaneously every 3 weeks. The rats received a primary boost of 50μg of rAd5 in 0.5ml RIBI followed by maintenance boosts of 10μg rAd5 in 0.5ml RIBI. The rabbits received 25μg of rAd5 in 1.0 ml RIBI

30   boosts throughout. Production bleeds were collected seven days post immunization for all animals. Animals were maintained and procedures preformed by Covance Research Products located in Denver, Pennsylvania.

1.8   Protocol for rAd5 Administration to Pigs and Humans

35   A total of six immune Yorkshire pigs received multiple subcutaneous and repeated intradermal injections of rAd5 for eight weeks. Cancer patients were administered rAd5 based on the type of cancer and the location of the tumor. All patients received at least 7.5×10^8 particles of rAd5 per dose. Patients with

Hepatocellular Carcinoma were administered a single dose of by percutaneous hepatic artery catheter over a 10 minute period. Patients with malignant head and neck cancer, breast cancer and non-small cell lung cancer received a single intratumoral injection. Cancer patients with peritoneal carcinomatosis from ovarian tumors received a single

5   intraperitoneal instillation over 20 minutes. Serum samples were collected at the indicated time points for analysis.

1.9   ELISA Procedure for Anti-rAd5 Antibodies

        Anti-rAd5 antibodies were measured using a sandwich ELISA . Prior to the

10   two-day sandwich ELISA, rAd5 (Schering-Plough, Kenilworth,N.J.) was coated onto microtiter platesovernight and then plates were blocked with bovine serum albumin (BSA). The presence of antibodies directed against rAd5 in pig serum samples or human serum was detected by first diluting the controls and unknown samples 1:40 into phosphate buffered saline (PBS) containing 1% BSA on the plate containing the

15   immobilized rAd5. The samples are then diluted serially 2-fold down the plate. After an overnight incubation, unreacted material was removed by washing the plates and a biotin-labeled Protein A/G conjugate was added for 2 hours and then the unbound conjugate was removed by washing. Horseradish peroxidase (HRP) conjugated streptavidin was then added for 2 hours. The bound HRP-conjugate was quantitatively

20   measured after addition of the enzyme's substrate, TMB (3,3',5,5' tetramethyl benzidine). The amount of antibody against rAd5 is proportional to the intensity of the colored end product of the enzymatic reaction. Samples are considered positive for the presence of antibodies to rAd5 if the mean of the sample O.D. / NPS O.D.is $\geq$0.1 for pig samples (established during assay validation) and O.D. / NHS O.D. is $\geq$ 0.28 for

25   human samples. In addition, samples were considered positive for development of antibodies after treatment with rAd5 if the mean O.D. of the post-dose sample was $\geq$ 2-fold the mean O.D. from the pre-dose sample obtained from the same pig or human serum sample.

30   2.0   Bioassay for Serum Neutralizing Factors

        Serum neutralizing antibodies (SNF) to a rAd5 were determined by using a SaOS-2 anti-proliferation assay. In this assay, the rAd5 vector expresses a p53 protein, a critical checkpoint in cell cycle regulation. The absence or mutation of p53 protein impairs the cell's ability to regulate proliferation resulting in uncontrolled growth. The

35   SaOS-2 cell line (human osteogenic sarcoma) lacks the p53 gene. Infection of SaOS-2 cells with rAd5 expressing p53 results in the inhibition of cell growth. The presence of SNFs will lead to a reduction in the degree of p53-induced inhibition of SaOS-2 proliferation. This is measured as a reduction in anti-proliferation activity of rAd5

spiked into a serum sample resulting in a shift of a dose response curve. The cellular response is monitored using the tetrazolium salt MTT, which is metabolized to formazan, solublized with sodium dodecyl sulfate (SDS) and then measured spectrophotometrically.

5   The observed optical density is related to log cell concentration. Neutralization activity of a serum sample is calculated by comparing the dilution corresponding to 50% maximal inhibition of SaOS-2 cell proliferation of a sample spiked with a standard amount of rAd5 to the dilution corresponding to 50% maximal inhibition of SaOS-2 cell proliferation by a standard amount of rAd5 in the absence of sample. Sample

10   results were expressed as % control. Sample results greater than 85 % of control were considered negative for SNF and sample results less than 85 % were reported as positive for SNF.

Table 1. Amino acid sequences of the seven peptides. Each peptide contains a common amino terminus cysteine residue directly followed by a KGKG linker. The remaining amino acid sequences correspond to the seven unique hypervariable regions (HVR) of adenovirus type 5 (Ad5) hexon.

5

Peptide Designation	Amino acid sequence corresponding to Ad5 hexon

10

	137 168
HVR1	CKGKGAATALEINLEEEDDDNEDEVDEQAEQQKTHVF

	185 197
HVR2	CKGKGIGVEGQTPKYADK

15

	210 225
HVR3	CKGKGYETEINHAAGRVLKK

	247 260
HVR4	CKGKGGILVKQQNGKLESQ

20

	267 283
HVR5	CKGKGSTTEATAGNGDNLTPKV

25

	302 315
HVR6	CKGKGMPTIKEGNSRELMG

	421 438
HVR7	CKGKGVINTETLTKVKPKTGQENGWEKDATEFSDK

30

Table 2. Antiserum Reactivity to Immomilized Peptides from Pig, Rat, and Rabbit Immunized with rAd5

Animal I.D.	Timepoint	Peptide:	Biosensor results in Response units (RU)						
			HVR1	HVR2	HVR3	HVR4	HVR5	HVR6	HVR7
PIG									
Ozzie	Pre		3	4	3	3	3	3	4
Ozzie (6 months post)	post		**456**	**209**	**141**	**578**	**212**	**143**	**205**
RAT									
Normal rat serum (NRS)	NRS		47	44	76	46	60.5	36	34
Pooled rat serum from six rats (12 month post)	post		**456**	25	105	**357**	24	**87**	**215**
RABBIT									
Normal rabbit serum (NRbS)	NRbS		9	7.5	ND	6.8	5.5	ND	ND
Rabbit 504 (17 months post)	post		**754**	**46**	**115**	**204**	**47**	**92**	**636***

pre = pre-dosed sample
post = post-dosed sample
ND = Not Determined
D = Day after dosing
* Although there is no data for NRbS, the sample was considered positive due to strong reactivity.

Positive results are indicated in **Bold** based on the following ratio:
RU of post sample / RU of pre,NRS or NRbS sample ≥ 2.0

Table 3. Reactivity of Human Clinical samples from Study C95-178 to Immobilized Peptides

Patient Initials	Timepoint	Peptide:	Biosensor results in Response units (RU)						
			HVR1	HVR2	HVR3	HVR4	HVR5	HVR6	HVR7
ER	PRE		19	19	38.4	14.1	22.1	21.9	28
	D7		ND	ND	ND	ND	ND	ND	ND
	D14		26.2	27.3	41	21.1	28.7	24	31
	D21		20.9	22.3	37	17.1	17.8	21	27
	PRE		23.6	26.9	49.8	25.3	26.1	72.5	29.8
FH	D3		21	21	ND	27	22.5	ND	ND
	D7		386	17.3	ND	20.4	15.5	ND	ND
	D14		1161	34.2	54.6	33.9	36.8	82.7	32.1
	D21		844.9	32.7	47.8	29.3	33.9	68	25
IK	PRE		24.2	19.9	37.4	12.4	28	17	21.3
	D7		2045	13.7	ND	41.6	15	ND	ND
	D14		8266	25.3	37.4	100.6	24.6	17	21.3
	D21		7549	13	ND	61	9	ND	ND
	D28		8385	29	38.9	87.3	36.5	18.4	23
	2MO.		2796	10	ND	18	8.5	ND	ND
HH	PRE		32.9	20.2	60.8	19.1	22.4	38.1	51
	D3		30.3	13	ND	13	5	ND	ND
	D7		60.4	22	44.6	22	18	28.3	42.5
MK	PRE		10.8	14.2	29.1	13.1	10.9	15.2	13.2
	2MO.		169.8	14.9	ND	9	5	13.2	38.4
	D28		433.3	25.2	41.1	17.9	20.4	27.2	ND
	D21		699	21.5	ND	15	9	ND	ND
	D14		659.6	22.6	ND	17.4	16	ND	13.7
	D7		ND	23.3	ND	13.1	15.9	15.5	13.2
	D3		ND	17.6	ND	ND	ND	ND	ND
SM	D7		14.2	ND	ND	15.5	ND	15.5	13.7
	PRE		23.3	23.3	37.3	16.2	27.3	16	13.2
	D6		108	20	40	14.1	31.5	19.5	1230
	D27		173	12.3	28.6	8.6	21	15.5	1026
	D48		150.8	7.4	29.2	4.4	10.7	19	522.5

D = Day
ND = Not Determined
pre = pre-dose

Table 4. Reactivity of Commercial Type-Specific Adenovirus Antibodies to Immobilized Peptides

Vendor	Ab Specificity	Sample I.D.	Biosensor results in Response units (RU)						
			Peptide: HVR1	HVR2	HVR3	HVR4	HVR5	HVR6	HVR7
Biogenesis	Goat anti-Ad2 hexon	post	25	3	17	3	5	15	15
Accurate	Murine anti-Ad penton (65 KD)	post	0	0	0	0	0	0	0
Biodesign	Goat anti-Ad2 hexon	post	30	10	ND	8	13	ND	ND
Biospecific	anti-Ad6	post	ND	ND	16	ND	ND	10	13
Biospecific	anti-Ad3	post	ND	ND	0	ND	ND	0	0
Lee Biol.	Rabbit anti-Ad5	pre	0	0	0	0	0	0	0
		post	255	0	6	0	0	8	10

pre = pre-dosed sample
post = post-dosed sample
ND = Not Determined

Positive results are indicated in **Bold** based on the following criteria:
1) Ratio of RU of post sample / RU of pre sample ≥ 2.0 OR
2) Post sample (without pre) with a binding greater than 50 RU

20

Table 5. Reactivity of Pre-Clinical Serum Samples to Immobilized Peptides from Pigs that Received rAd5

			Biosensor results in Response units (RU)						
Sample I.D.	Timepoint	Peptide:	HVR1	HVR2	HVR3	HVR4	HVR5	HVR6	HVR7
377	pre		66	46	101	39	41	87	139
5	D15		**1085**	58	112	79	58	108	151
384	pre		73	71	99	52	57	98	129
8	D15		**795**	57	87	**131**	51	70	102
8	D29		**1620**	65	81	**111**	62	71	101
393	pre		96	199	83	83	100	65	80
12	D15		**483**	127	71	140	57	95	72
12	D29		**1490**	107	92	**201**	63	104	99
12	D48		**665**	113	107	**177**	94	86	91
389	pre		48	45	73	34	48	69	87
11	D15		**729**	39	52	32	50	53	64
11	D29		**1494**	61	62	**125**	68	58	80
11	D48		**1024**	66	61	**136**	65	54	84
390	pre		72	80	74	77	73	60	94
7	D15		**936**	64	70	62	56	60	81
7	D29		**1200**	66	69	75	60	58	76
385	pre		107	80	65	62	72	59	81
3	D15		**567**	49	41	41	42	40	55

pre = pre-dose sample
D = Day after dosing
Positive results are indicated in **Bold** based on the following ratio:
RU of post sample / RU of pre sample $\geq$ 2.0

Table 6. Reactivity of Serum Samples to Immobilized Peptides from Patients with Non-Small Cell Lung Cancer Administered rAd5

Patient Initials	Timepoint	Peptide:	Biosensor results in Response units (RU)						
			HVR1	HVR2	HVR3	HVR4	HVR5	HVR6	HVR7
ER	PRE		19	19	38	14	22	22	28
	D7		ND	ND	ND	ND	ND	ND	ND
	D14		26	27	41	21	29	24	31
	D21		21	22	37	17	18	21	27
FH	PRE		24	27	50	25	26	73	30
	D3		21	21	ND	27	23	ND	ND
	D7		386	17	ND	20	16	ND	ND
	D14		1161	34	55	34	37	83	32
	D21		845	33	48	29	34	68	25
IK	PRE		24	20	37	12	28	17	21
	D7		2045	14	ND	**42**	15	ND	21
	D14		8266	25	37	**101**	25	17	21
	D21		7549	13	ND	**61**	9	ND	ND
	D28		8385	29	39	**87**	37	18	23
	2MO.		2796	10	ND	18	9	ND	ND
HH	PRE		33	20	61	19	22	38	51
	D3		30	13	ND	13	5	ND	ND
	D7		60	22	ND	22	18	ND	ND
	D14		660	23	45	17	16	28	43
	D21		699	22	ND	15	9	ND	ND
	D28		433	25	41	18	20	27	38
	2MO.		170	15	ND	9	5	ND	ND
MK	PRE		11	14	29	13	11	15	13
	D3		ND	ND	ND	ND	ND	ND	ND
	D7		14	18	29	16	16	16	14
SM	PRE		23	23	37	16	32	16	1230
	D6		108	20	40	14	27	20	1026
	D27		173	12	29	9	21	19	523
	D48		151	7	ND	4	11	ND	ND

D = Day after dosing
ND = Not Determined
pre = pre-dose
MO. = Month's after dosing
Positive results are indicated in **Bold** based on the following ratio:
RU of post sample / RU of pre sample ≥ 2.0

Table 7. Reactivity of Clinical Samples to Immobilized Peptide from Patients Administered rAd5

A. Patients with Recurrent & Malignant Head and Neck Cancer

Patient Initials	Timepoint	Peptide:	HVR1	HVR3	HVR4	HVR7
TGL	PRE		11	32	17	15
	D3		9	24	12	12
	D8		10	29	14	13
	D15		9	27	14	13
	D22		11	31	16	16
	D28		11	29	16	17
RWT	PRE		53	59	27	32
	D3		47	50	23	28
	D10		58	52	28	36
	D14		42	35	17	23

B. Patient with Recurrent Melanoma & Breast Cancer

Patient Initials	Timepoint	Peptide:	HVR1	HVR3	HVR4	HVR7
ES	PRE		27	27	24	20
	D3		23	26	20	18
	D7		54	27	23	19
	D14		**252**	28	22	19
	D21		**185**	28	21	18
	D28		**79**	15	10	8

C. Patient with Peritoneal Carcinomatosis

Patient Initials	Timepoint	Peptide:	HVR1	HVR3	HVR4	HVR7
VKA	PRE		7	35	16	12
	D2		10	33	17	16
	D3		8	30	13	17
	D7		14	49	26	17
	D14		41	36	16	**29**
	D21		**45**	44	25	**26**
	D28		**38**	31	16	**25**

D. Patients with Hepatocellular Carcinoma

Patient Initials	Timepoint	Peptide:	HVR1	HVR3	HVR4	HVR7
JT	PRE		50	206	40	132
	D2		10	33	17	16
	D3		25	136	18	85
	D7		**1629**	109	27	73
	D14		**7538**	171	**108**	111
	D21		**5208**	187	**102**	116
	D28		**3837**	174	**83**	108
WMF	PRE		17	24	20	16
	D3		17	23	20	18
	D7		**68**	18	17	15
	D14		**163**	27	22	21
	D28		**106**	19	18	19
DFB	PRE		12	21	13	14
	D3		14	20	14	18
	D7		**49**	15	12	16
	D14		**1226**	26	14	19
	D21		**606**	22	10	14
	D28		**414**	32	19	23
RK	PRE		21	21	14	19
	D3		19	18	12	19
	D7		40	25	20	25
	D14		**113**	21	15	18
	D21		**75**	19	13	19
	D28		**88**	32	22	32

PRE = pre-dose
D = Day after dosing
Positive results are indicated in **Bold** based on the following ratio:
RU of post sample / RU of pre sample $\geq$ 2.0

Table 8. Reactivity of pre-clinical swine serum samples from SN 96380 to Immobilized Peptides

Sample I.D.	Timepoint	Peptide	Biosensor results in Response units (RU)						
			HVR1	HVR2	HVR3	HVR4	HVR5	HVR6	HVR7
377	pre		66.4	46.2	100.8	38.9	41	87.3	138.5
5	D15		1085	57.6	111.9	79	58	107.6	150.6
384	pre		73.2	71.4	98.9	52.3	57.3	97.6	129.4
8	D15		794.9	56.6	87	130.6	50.7	70	102.3
8	D29		1620	65	81	111.4	62.3	71	101
393	pre		96	199	83.4	83	100.4	65	80
12	D15		482.7	127	71	140	57.2	95	72
12	D29		1490	106.5	92	200.8	63	103.5	99.1
12	D48		664.6	113.2	106.8	176.5	94	86	91
389	pre		47.7	44.6	72.5	33.5	47.9	69	87
11	D15		728.6	39	52	32	50	52.5	64
11	D29		1494	61.4	62	124.8	68.3	58	80
11	D48		1024	65.6	61	136.2	64.9	54	84
390	pre		71.6	80	73.6	77.3	72.9	60	94
7	D15		936	64	69.5	62	55.6	60	80.5
7	D29		1200	66.4	69	75	60	58	76
385	pre		106.7	80	65	62	72	59	81
3	D15		567.4	49.1	41	41	42	40	55

pre = pre-dose sample
D = Day

Table 9.
C95-177-01- RECURRENT OR METASTATIC HEAD & NECK CANCER

INITIALS	SAMPLE	HVR1	HVR3	HVR4	HVR7
TGL	PRE	11.3	31.5	16.6	15.2
	D3	8.7	23.6	12.1	12.4
	D8	9.5	28.9	14	13.4
	D15	9.2	27	13.6	13.3
	D22	11	30.7	16.3	15.5
	D28	10.9	29.3	15.6	16.6
RWT	PRE	52.5	59	26.6	31.8
	D3	46.8	49.8	23	28.3
	D10	58	52.3	27.9	36.3
	D14	41.5	35	17.4	22.9

I95-082-04 - RECURRENT MELANOMA OR BREAST CANCER

INITIALS	SAMPLE	HVR1	HVR3	HVR4	HVR7
ES	PRE	27	27.4	23.5	19.8
	D3	22.8	25.8	20.3	17.8
	D7	53.7	27.4	23.2	19.4
	D14	252	28.2	21.7	18.6
	D21	185	27.6	20.8	17.7
	D28	78.6	14.6	10	8.2

C95-084-01 - PERITONEAL CARCINOMATOSIS

INITIALS	SAMPLE	HVR1	HVR3	HVR4	HVR7
VKA	PRE	7.4	35	16.2	12
	D2	10.1	33	16.9	15.6
	D3	7.7	29.7	13.1	16.5
	D7	14	48.6	26	17.4
	D14	41	36	16	28.9
	D21	44.5	43.8	25.4	26.1
	D28	38.1	30.6	16.3	24.7

C95-063-01 – HEPATOCELLULAR CARCINOMA

INITIALS	SAMPLE	HVR1	HVR3	HVR4	HVR7
CLL	PRE	8.3	15.9	12.6	11.7
	D3	6.8	17.8	11.8	10.4
JT	PRE	50.3	205.8	39.7	131.7
	D2	10.1	33	16.9	15.6
	D3	24.6	135.6	18	85.1
	D7	1629	108.5	26.6	72.6
	D14	7538	170.6	108	110.6
	D21	5208	186.5	102	116.1
	D28	3837	173.8	82.5	107.8
EL	PRE	23.4	26.6	21.4	22.4
JK	PRE2	21.7	33.7	20.6	33.8
	PRE1	17	28.8	15.9	26.5
WMF	PRE	16.7	23.5	19.5	15.8
	D3	17.3	22.5	19.5	17.5
	D7	67.7	18.3	17	15.3
	D14	163	26.8	22.3	21.4
	D28	106	19.2	17.7	18.9
DFB	PRE	12.3	21	12.6	14
	D3	13.7	20.3	13.9	18.1
	D7	48.7	15.3	11.5	15.6
	D14	1226	26.3	14.3	18.6
	D21	606	22.3	10.4	14.2
	D28	414	32.3	19.1	22.6
RK	PRE	20.7	20.5	14.2	19.2
	D3	18.5	17.5	12.4	19.4
	D7	39.5	24.6	19.5	24.6
	D14	113	21.2	14.9	18
	D21	75.3	19	13.1	19.3
	D28	87.6	31.9	21.9	31.8

Table 10. Results of Anti-rAd5 Serum Antibody Determined by ELISA, Bioassay and Biosensor Assay from Cancer Patients Administered rAd5

Cancer Diagnosis	# of Patients	Sample I.D.	Ratio of # Positive Ab / Total # samples		
			ELISA	Bioassay	Biosensor
NSCLC	6	pre	6 / 6	5 / 6	0 / 6
		post	4 / 6	1 / 6	4 / 6
Melanoma and Breast Cancer	1	pre	0 / 1	0 / 1	0 / 1
		post	1 / 1	1 / 1	1 / 1
Malignant Head and Neck Cancer	2	pre	2 / 2	2 / 2	0 / 2
		post	0 / 2	0 / 2	0 / 2
Peritoneal Carcinomatosis	1	pre	1 / 1	1 / 1	0 / 1
		post	1 / 1	0 / 1	1 / 1
Hepatocellular Carcinoma	4	pre	4 / 4	4 / 4	0 / 4
		post	2 / 4	0 / 4	4 / 4

pre = pre-dosed sample
post = post-dosed sample
ND = Not Determined

Positive results are indicated in **Bold** based on the following criteria:
1) Ratio of RU of post sample / RU of pre sample ≥ 2.0 OR
2) Post sample (without pre) with a binding greater than 50 RU

Table 11. Summary of Results for Anti-rAd5 Serum Antibody Determined by ELISA, Bioassay and Biosensor Assay from Cancer Patients Administered rAd5

	# Ab Positive / # samples		
	ELISA	Bioassay	Biosensor
Presence of pre-existing Anti-Ad5 Abs	14 / 14	12 / 14	0 / 14
Development of Anti-Ad5 Abs	7 / 14	2 / 14	10 / 14

ELISA Antigen = rAd5
Biosensor Antigen = specific Ad5 peptides HVR1,3,4,and 7
Bioassay = rAd5 infection of cells

29

From the above, it is clear that the present invention provides an efficient method of determining the likelihood that a patient host's immune system will interfere with intended gene therapy using viral vectors.

```
                              SEQUENCE LISTING

   SEQ ID NO: 1 is a peptide sequence for the detection of adenovirus
   SEQ ID NO: 2 is a peptide sequence for the detection of adenovirus
   SEQ ID NO: 3 is a peptide sequence for the detection of adenovirus
   SEQ ID NO: 4 is a peptide sequence for the detection of adenovirus
   SEQ ID NO: 5 is a peptide sequence for the detection of adenovirus
   SEQ ID NO: 6 is a peptide sequence for the detection of adenovirus
   SEQ ID NO: 7 is a peptide sequence for the detection of adenovirus
   SEQ ID NO: 8 is a peptide sequence for the detection of adenovirus


   <110> Mytych, Daniel

   <120> METHODS AND REAGENTS FOR THE DETECTION OF ANTIBODIES TO
         ADENOVIRUS

   <130> JB0976

   <140>
   <141>

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   <170> PatentIn Ver. 2.1

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   <213> Artificial Sequence

   <220>
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         detection of adenovirus

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   Glu Asp Glu Val Asp Glu Gln Ala Glu Gln Gln Lys Thr His Val Phe
                    20                  25                  30




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Val

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<213> Artificial Sequence

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 1   5   10   15

Glu Asn Gly Trp Glu Lys Asp Ala Thr Glu Phe Ser Asp Lys
             20   25   30

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<223> Description of Artificial Sequence: Peptide for
      detection of adenovirus

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Cys Lys Gly Lys Gly
 1   5

32
CLAIMS

We claim:

5   1.   A method for detecting antibodies capable of binding to adenovirus
comprising:
         a)   immobilization of a peptide capable of being bound by an anti-
                 adenovirus antibody directly onto a flowcell of a sensorchip in a
                 biosensor,
10   b)   obtaining a serum sample from a patient to be tested and contacting
                 said serum sample with the immobilized peptide, and
         c)   measuring binding of antibodies to the immobilized peptide by means
                 of response units from said biosensor.

15 2.   The method of Claim 1, wherein said peptide is capable of being bound by
antibodies specific to adenovirus 5.

    3.   The method of Claim 2, wherein said serum is human serum.

20 4.   The method of Claim 3, wherein said peptide is selected from the group
consisting of:
         a)   a peptide comprising SEQ ID NO: 1, or a peptide having substantial
                 sequence identity thereto;
         b)   a peptide comprising SEQ ID NO: 2, or a peptide having substantial
25   sequence identity thereto;
         c)   a peptide comprising SEQ ID NO: 3, or a peptide having substantial
                 sequence identity thereto;
         d)   a peptide comprising SEQ ID NO: 4, or a peptide having substantial
                 sequence identity thereto;
30   e)   a peptide comprising SEQ ID NO: 5, or a peptide having substantial
                 sequence identity thereto;
         f)   a peptide comprising SEQ ID NO: 6, or a peptide having substantial
                 sequence identity thereto; and

g) a peptide comprising SEQ ID NO: 7, or a peptide having substantial sequence identity thereto.

5. The method of Claim 4, wherein said peptide is selected from the group consisting of:

a) a peptide comprising SEQ ID NO: 9, or a peptide having substantial sequence identity thereto;

b) a peptide comprising SEQ ID NO: 10, or a peptide having substantial sequence identity thereto;

c) a peptide comprising SEQ ID NO: 11, or a peptide having substantial sequence identity thereto;

d) a peptide comprising SEQ ID NO: 12, or a peptide having substantial sequence identity thereto;

e) a peptide comprising SEQ ID NO: 13, or a peptide having substantial sequence identity thereto;

f) a peptide comprising SEQ ID NO: 14, or a peptide having substantial sequence identity thereto; and

g) a peptide comprising SEQ ID NO: 15, or a peptide having substantial sequence identity thereto.

6. The method of Claim 5, wherein the amount of antibody that binds to each peptide is directly proportional to said response units.

7. The method of Claim 6 wherein said response is detected using a BIACORE 2000™.

8. The method of Claim 1 further comprising a plurality of peptides capable of being bound by an anti-adenovirus antibody are directly immobilized, each on its own separate flowcell.

9. The method of Claim 8, wherein at least one of said plurality of peptides is capable of being bound by antibodies specific to adenovirus 5.

10. The method of Claim 9, wherein said serum is human serum.

11. The method of Claim 10, wherein each of said plurality of peptides are selected from the group consisting of:

5
     a) a peptide comprising SEQ ID NO: 1, or a peptide having substantial sequence identity thereto;

     b) a peptide comprising SEQ ID NO: 2, or a peptide having substantial sequence identity thereto;

     c) a peptide comprising SEQ ID NO: 3, or a peptide having substantial

10
     sequence identity thereto;

     d) a peptide comprising SEQ ID NO: 4, or a peptide having substantial sequence identity thereto;

     e) a peptide comprising SEQ ID NO: 5, or a peptide having substantial sequence identity thereto;

15
     f) a peptide comprising SEQ ID NO: 6, or a peptide having substantial sequence identity thereto; and

     g) a peptide comprising SEQ ID NO: 7, or a peptide having substantial sequence identity thereto.

20
12. The method of Claim 11, wherein said peptide is selected from the group consisting of:

     a) a peptide comprising SEQ ID NO: 9, or a peptide having substantial sequence identity thereto;

     b) a peptide comprising SEQ ID NO: 10, or a peptide having substantial

25
     sequence identity thereto;

     c) a peptide comprising SEQ ID NO: 11, or a peptide having substantial sequence identity thereto;

     d) a peptide comprising SEQ ID NO: 12, or a peptide having substantial sequence identity thereto;

30
     e) a peptide comprising SEQ ID NO: 13, or a peptide having substantial sequence identity thereto;

     f) a peptide comprising SEQ ID NO: 14, or a peptide having substantial sequence identity thereto; and

35

g)   a peptide comprising SEQ ID NO: 15, or a peptide having substantial sequence identity thereto.

13.   The method of Claim 12 wherein the serum sample is simultaneously contacting with a plurality of immobilized peptides.

14.   The method of Claim 13 wherein the amount of antibody that binds to each peptide is directly proportional to said response units.

15.   The method of Claim 14 wherein said biosensor is a BIACORE 2000™.

16.   A method for detecting antibodies capable of binding to adenovirus, comprising a peptide selected from the group consisting SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7 or a peptides having substantial sequence identity thereto.

17.   The method of Claim 16, wherein said method of detecting comprises an ELISA system.

18.   The method of Claim 17, further comprising a plurality of peptides capable of being bound by an anti-adenovirus antibody.

19.   A composition of matter comprising SEQ ID No. 8 or a peptide having substantial sequence identity thereto.

20.   The use of the composition of matter of Claim 19 in a biosensor based assay to detect antibodies.

ABSTRACT

METHODS AND REAGENTS FOR THE DETECTION
OF ANTIBODIES TO ADENOVIRUS

5

The present invention provides methods and reagents for detecting antibodies to adenovirus. In a preferred embodiment, the present invention is useful for detecting antibodies to adenovirus serotype 5. In a further preferred embodiment, the present invention can be used in a biosensor-based assay. It is contemplated that the

10   present invention is useful to detect antibodies for ascertaining adenovirus infection, evaluating patient response to gene therapy using adenovirus vectors, developing vaccines to adenovirus infection, developing therapeutics for inducing passive immunity to adenovirus infection, as well as other uses.

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)	Attorney Docket Number	JB0976Q
	First Named Inventor	Mytych
	COMPLETE IF KNOWN	
☒ Declaration Submitted with Initial Filing **OR** ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)	Application Number	/
	Filing Date	August 22, 2000
	Group Art Unit	
	Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

> METHODS AND REAGENTS FOR THE DETECTION OF ANTIBODIES TO ADENOVIRUS

the specification of which *(Title of the Invention)*

☒ is attached hereto
OR
☐ was filed on (MM/DD/YYYY) [   ] as United States Application Number or PCT International

Application Number [   ] and was amended on (MM/DD/YYYY) [   ] (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	
60/150,622	08/25/99	☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

[Page 1 of 2]

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U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

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Name	Registration Number	Name	Registration Number
Donald W. Wyatt	40879		

☒ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: ☐ Customer Number or Bar Code Label [24265] OR ☒ Correspondence address below

Name	Donald W. Wyatt
Address	Patent Dept. K-6-1, 1990
Address	2000 Galloping Hill Road

City	Kenilworth	State	NJ	ZIP	07033-0530
Country	USA	Telephone	(908) 298-2902	Fax	(908) 298-5388

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Name of Sole or First Inventor:	☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Daniel T.	Mytych

Inventor's Signature		Date	

Residence: City	Thousand Oaks	State	CA	Country	U.S.A.	Citizenship	U.S.A.

Post Office Address	3162 Saddleback Court
Post Office Address	

City	Thousand Oaks	State	CA	ZIP	91360	Country	U.S.A.

☒ Additional inventors are being named on the __1__ supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Steven J.	Swanson

Inventor's Signature		Date	
Residence: City Moorpark	**State** CA	**Country** U.S.A.	**Citizenship** U.S.A.

Post Office Address 4318 Timberdale Road

Post Office Address

City Moorpark	**State** CA	**ZIP** 93021	**Country** U.S.A.

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname

Inventor's Signature		Date	
Residence: City	**State**	**Country**	**Citizenship**

Post Office Address

Post Office Address

City	**State**	**ZIP**	**Country**

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname

Inventor's Signature		Date	
Residence: City	**State**	**Country**	**Citizenship**

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Name	Registration Number	Name	Registration Number
Margaret M. Albanese	45525	Susan Lee	30653
Edwin P. Ching	34090	Anita W. Magatti	29825
Nancy V. Connelly	46638	Arthur Mann	35598
Eric S. Dicker	31699	Christine F. Martin	39762
Robert A. Franks	28605	Edward H. Mazer	27573
James M. Gould	33702	Richard B. Murphy	35296
Richard J. Grochala	31518	James R. Nelson	27929
Henry S. Hadad	35888	Pamela G. Salkeld	38607
Thomas D. Hoffman	28221	David B. Schram	43096
Henry C. Jeanette	30856	Immac J. Thampoe	36322
Palaiyur S. Kalyanaraman	34634	Paul A. Thompson	35385
Gerald P. Keleher	43707	Donald W. Wyatt	40879
Allan N. Kutzenco	38945	Sandy Zaradic	45997

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